

**Amendments to the claims:**

Claim 1 (Currently Amended): A method for differentiating one or more pluripotent mammalian embryonic stem (ES) cells comprising:

- a. culturing the mammalian ES cells at low density in a serum-free and feeder-layer free media ~~optionally~~ comprising leukemia inhibitory factor; and
- b. allowing said mammalian ES cells to differentiate to primitive neural stem cells.

Claim 2 (Currently Amended): The method according to claim 1 for differentiating mammalian embryonic stem cells to cells with markers characteristic of neural cells comprising:

- a. culturing the mammalian embryonic stem cells in the serum free and feeder-layer free media at low cell density wherein said density is selected to minimize ES cell aggregation or EB formation; and
- b. allowing said cells to differentiate.

Claim 3 (Previously Presented): The method of claim 2 wherein the cell density is selected as to avoid EB formation.

Claim 4 (Previously Presented): The method of claim 1 wherein said cell density falls within the range of greater than 0 cells/ $\mu$ l to less than or equal to 50 cells/ $\mu$ l.

Claim 5 (Previously Presented): The method of claim 4 wherein the cell density falls within the range of greater than 0 cells/ $\mu$ l to less than or equal to 20 cells/ $\mu$ l.

Claim 6 (Previously Presented): The method of claim 5 wherein the cell density falls within the range of greater than 0 cells/ $\mu$ l to less than or equal to 10 cells/ $\mu$ l.

Claim 7 (Original): The method of claim 6 wherein the cell

density is 10 cells/ $\mu$ l.

Claim 8 (Currently Amended): The method of ~~claims~~ claim 6 wherein there is no EB formation.

Claim 9 (Previously Presented): The method of claim 7 wherein the differentiating ES cells form at least one sphere colony.

Claim 10 (Currently Amended): The method of claim 1 wherein the differentiating mammalian ES cells form at least one sphere colony.

Claim 11 (Original): The method of claim 1 wherein the serum free media further comprises a cytokine.

Claim 12 (Cancelled)

Claim 13 (Previously Presented): The method of claim 1 wherein the primitive neural stem cells are pluripotent.

Claim 14 (Previously Presented): The method of claims 1 wherein the serum free media further comprises a growth factor.

Claim 15 (Original): The method of claim 14 wherein the growth factor is selected from the members of the fibroblast growth factor (FGF) family of growth factors.

Claim 16 (Original): The method of claim 15 wherein the growth factor is FGF2.

Claim 17 (Previously Presented): The method according to claim 1 wherein the media comprises Noggin or a compound from the Cerberus family of proteins.

Claim 18 (Cancelled)

Claim 19 (Cancelled)

Claim 20 (Currently Amended): A method for producing secondary mammalian primitive neural stem cell colonies comprising:

- a. culturing mammalian ES cells in low cell density serum-free and feeder-layer free media ~~optionally~~ comprising leukemia inhibitory factor for a time and under conditions sufficient to differentiate the said mammalian ES cells to primary primitive neural stem cell colonies;
- b. dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and
- c. administering a growth factor or survival factor to the dissociated neural cells to produce secondary primitive neural stem cell colonies.

Claim 21 (Original): A method according to claim 20 wherein the growth factor is selected from among the members of the fibroblast growth factor (FGF) family of growth factors.

Claim 22 (Original): A method according to claims 21 wherein the growth factor is FGF2.

Claims 23-32 (Cancelled)

Claim 33 (Currently Amended): A method for screening for modulators of mammalian primitive neural stem cell differentiation comprising:

- a. culturing mammalian primitive neural stem cells in serum-free and feeder-layer free media ~~optionally~~ comprising leukemia inhibitory factor under low density conditions in the presence of the potential

- modulator under conditions that produce differentiation in the absence of the modulator;
- b. detecting any differentiation of the cells and cell types generated, if any, in the presence of the modulator compared to differentiation and cell types generated in the absence of the modulator;
  - c. determining whether the modulator affects the differentiation of the cells.

Claim 34 (Original): A method in accordance with claim 33, wherein the modulators comprise any culturing conditions that may modulate cellular differentiation.

Claim 35 (Currently Amended): A method for screening for differentiation factors of cellular development comprising:

- a. culturing mammalian pluripotent embryonic stem (ES) cells in serum free media ~~optionally~~ comprising leukemia inhibitory factor at low cell density in the presence of the differentiation factor;
- b. allowing the cells to differentiate;
- c. detecting differentiation of the cells, if any.

Claim 36 (Previously Presented): A method of claim 35 further comprising determining whether the differentiation of the cells comprises neural cell development.

Claim 37 (Currently Amended): A method for screening for differentiation factors of cellular development comprising:

- a. culturing the primitive neural stem cells produced by the method of claim 1 29 in serum free media ~~optionally~~ comprising leukemia inhibitory factor, in the presence of the differentiation factor.
- b. detecting any differentiation of the cells.

Claim 38 (Original): The method of claim 37, wherein the media

further comprises FGF2.

Claim 39 (Cancelled)

Claim 40 (Cancelled)

Claim 41 (Previously Presented): The method of claim 1 further comprising determining whether the cells differentiate into a homogenous uniform cell base.

Claim 42 (Currently Amended): The method of claim 1 ~~29~~ further comprising determining whether the cells differentiate into a neural cell base.

Claims 43-46 (Cancelled)

Claim 47 (Currently Amended): A method for producing secondary mammalian primitive neural stem cell colonies comprising:

- a. culturing mammalian ES cells in low cell density serum-free and feeder-layer free media ~~optionally~~ comprising leukemia inhibitory factor for a time and under conditions sufficient to differentiate the said mammalian ES cells to primary primitive neural stem cell colonies;
- b. dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and
- c. administering LIF or B27 to the dissociated neural stem cells to produce secondary primitive neural stem cell colonies.

Claims 48-55 (Cancelled)

Claim 56 (Previously Presented): The method of claim 1, wherein said primitive neural stem cell expresses at least one

gene selected from the group consisting of nestin, GATA4, Emx2, and HoxB1.

Claims 57-59 (Cancelled)